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Cascade Systems in ω -Transaminase Reactions

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Production of chiral amines using transaminases has recently been reported as an interesting alternative to present organic synthesis methods. The chiral amines are building blocks for many new pharmaceuticals and two biocatalytic methods of production have been demonstrated: kinetic resolution and asymmetric synthesis. The latter approach has the advantage over existing biocatalytic and chemical methods that the theoretical yield is 100% compared to 50%. A major challenge of this approach is the unfavourable thermodynamic equilibrium which needs to be improved in order to meet the criteria of technical feasibility of biocatalytic process. This can be achieved by removing the product or co-product formed. For example, if 2-propylamine is used as amine donor, evaporation of formed acetone can be used. However, this approach is limited by the selectivity of the separation method.

An alternative for shifting equilibrium is by degrading or recycling one of the reaction co-products *in-situ* using additional enzymatic reactions. These cascade systems have been shown to be feasible at micro-litre scale, but still systems need to be characterized and be shown to be scalable and economically feasible in order to be industrially implemented.

The four selected cascades systems to be investigated are lactate dehydrogenase (EC 1.1.1.27)/glucose dehydrogenase (EC 1.1.99.10), alanine dehydrogenase (EC 1.4.1.1)/glucose dehydrogenase (EC 1.1.99.10), pyruvate decarboxylase (EC 4.1.1.1) and acetolactate synthase (EC 2.2.1.6). The aim of this work is to determine the effectiveness and constraints of these co-product degradation systems at process relevant conditions. An interaction matrix is suggested to describe the effects of the compounds involved in the process on the enzymes present in the reaction mixture. Further, the determination of kinetic parameters and establishment of kinetic mathematical model will help in future work of reactor selection and overall better understanding of this one-pot multi-enzyme system.